SCANNING ELECTRON MICROSCOPY OF SOYBEANS, SOY FLOURS, PROTEIN CONCENTRATES, AND PROTEIN ISOLATES[1]

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ABSTRACT

Soybean cotyledon fracture surfaces prepared by freezing in liquid nitrogen and samples of commercial soy flours, protein concentrates, and protein isolates were examined in the scanning electron microscope. Protein bodies and spherosomes characteristic of the native cellular structure were clearly discerned in the fracture surfaces and were also observed in a full-fat flour. Defatted flours likewise contained protein bodies; the largest number occurred in an unheated flour and the fewest were seen in a toasted flour. Protein concentrate made by alcohol leaching contained protein bodies, whereas a concentrate prepared by acid leaching consisted of partially collapsed spheres. The latter probably formed during spray drying of the neutralized concentrate. Isoelectric isolate particles were rough in surface texture and proteinate forms of isolates were smooth, apparently as a result of differences in solubility during spray drying.

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[2]Mention of firm names or trade products does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

We have recently described the advantages and usefulness of scanning electron microscopy for probing the subcellular structure of soybeans (1). Others have used this technique to a limited extent to examine soybean protein products. For example, Mustakas *et al.* (2,3) examined full-fat soy flour made by extrusion cooking and a beverage base made from the flour. Stanley *et al.* (4) studied spun

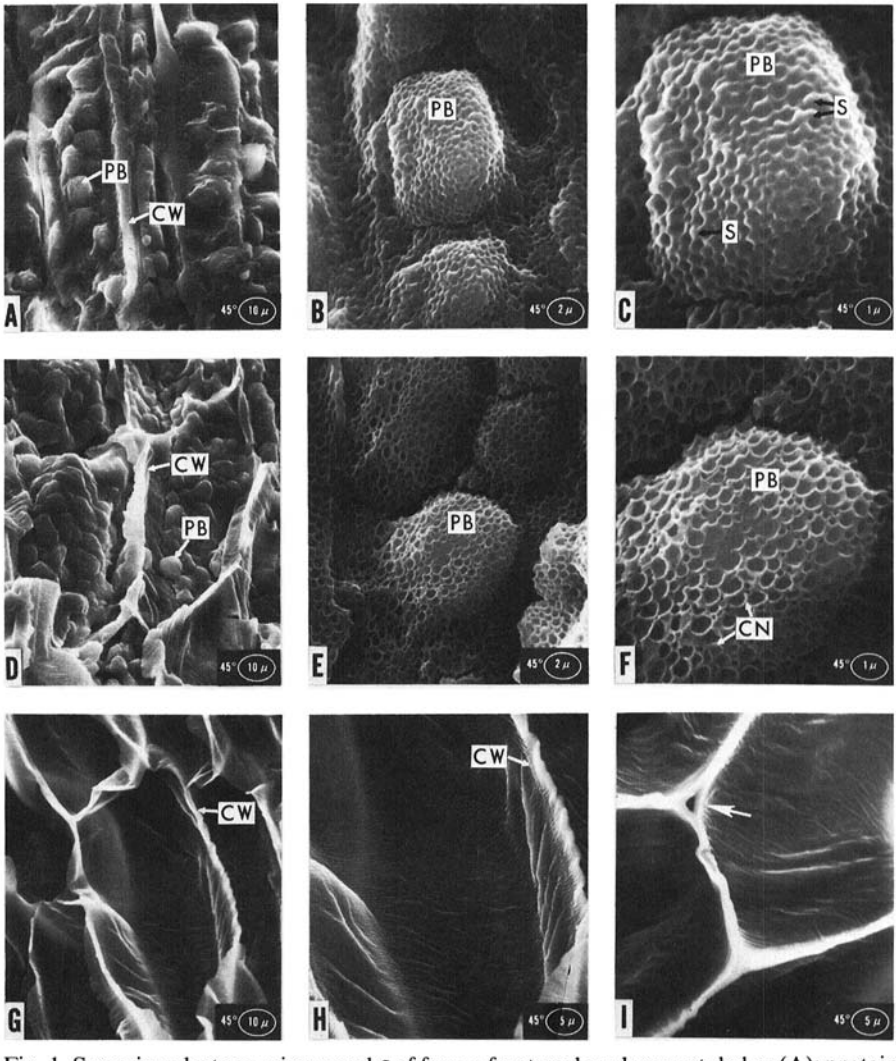

Fig. 1. Scanning electron micrographs of freeze-fractured soybean cotyledon (**A**); protein body covered with sponge-like cytoplasmic network and spherosomes in fracture surface (**B**, **C**); hexane defatted fracture surface (**D**); protein body in defatted fracture surface showing cytoplasmic network (**E**, **F**); and fracture surface showing empty cells after washing with water for 1 min (**G**, **H**, **I**). Cell wall (**CW**), protein body (**PB**), spherosomes (S), and cytoplasmic network (**CN**) are identified. Arrow in **I** points to juncture of three cells.

soy protein fibers while Cumming and coworkers (5) described scanning studies of experimentally extruded soybean meal. We have now extended our earlier work on the cellular structure and have also examined a variety of commercially available soybean protein products. Here we describe our findings with flours, concentrates, and isolates.

Samples and Specimen Preparation

Freeze-fractured surfaces (6) of soybean cotyledons (Beeson variety) were prepared by freezing cotyledons in liquid nitrogen (−196° C) and then dropping them onto a stone laboratory bench top, thereby shattering them. Some of the fracture surfaces were mounted and examined after coating with metal. Other fracture surfaces were washed several times with hexane to remove oil from the surface spherosomes before mounting and examining. Full-fat and defatted fracture surfaces were also washed by swirling the fractured cotyledons briefly in water, blotting, and air drying before examination.

Soybean flours, concentrates, and isolates were obtained from industrial concerns.

Samples of large-particle size were mounted on the specimen holders with plastic cement; small particles were sprinkled onto specimen holders covered with double-coated adhesive tape or with silver conductive paint (GC Electronics, Rockford, Ill.) while the paint was still tacky. All samples were coated with gold:palladium (60:40) and then examined in a Stereoscan Mark 2A scanning electron microscope (Cambridge Instrument Co., Ltd., London, England). The incident angle of the scanning electron beam to the surface of the specimen was 45°.

Cellular Structure of Soybean Cotyledons

Previously we reported on the structure of soybean cotyledons as observed primarily in mechanical fracture surfaces (1). We have now also examined soybean cotyledon surfaces prepared by "freeze-fracturing" (6) to obtain a greater insight into the cellular structure. Two major organelles, the protein bodies and the spherosomes, are the respective storage sites of protein and oil and are embedded in a cytoplasmic network. Figure 1A is an electron micrograph of a "freeze-fractured" cotyledon in which the cell wall and protein bodies are identified. Higher magnification shows that the spherosomes and cytoplasmic network cover the surface of the protein bodies and the interior cell wall surface. Figures 1B and 1C show enlarged views of a protein body covered with cytoplasmic network in which the spherosomes are embedded. Depressions (0.3–0.4 μ in diameter) in the spherosome-cytoplasmic network likely correspond to sites occupied by spherosomes before fracturing of the cotyledon. The missing spherosomes apparently remained attached to the other fracture surface.

Washing the fracture surface with hexane removes the oil in the spherosomes and more clearly reveals the protein bodies (Fig. 1D). Figure 1E and 1F show a cratered surface of a protein body and the craters are of two sizes. The largest pits (~0.4 μ in diameter) may correspond to similar-sized depressions noted in the undefatted surface (Figs. 1B and 1C). The smaller craters (~0.1 μ in diameter) are likely to be sites occupied by spherosomes before defatting with hexane. The cratered residue on the protein body presumably is the cytoplasmic network.

In our earlier studies we found that the cytoplasmic network on the protein body surfaces disappears when the protein bodies in defatted soy flour are isolated in aqueous sucrose buffered at pH 5 (1). Presumably, the meshlike network dissolves. We tested this conclusion by washing the fractured cotyledons in water for 1 min. Examination of the undefatted fracture surface showed that the cellular contents were completely removed (Fig. 1G). Higher magnification revealed only the wrinkled interior surface of the cell wall (Fig. 1H) and identical results were obtained with defatted fracture surfaces (Fig. 1I). These results

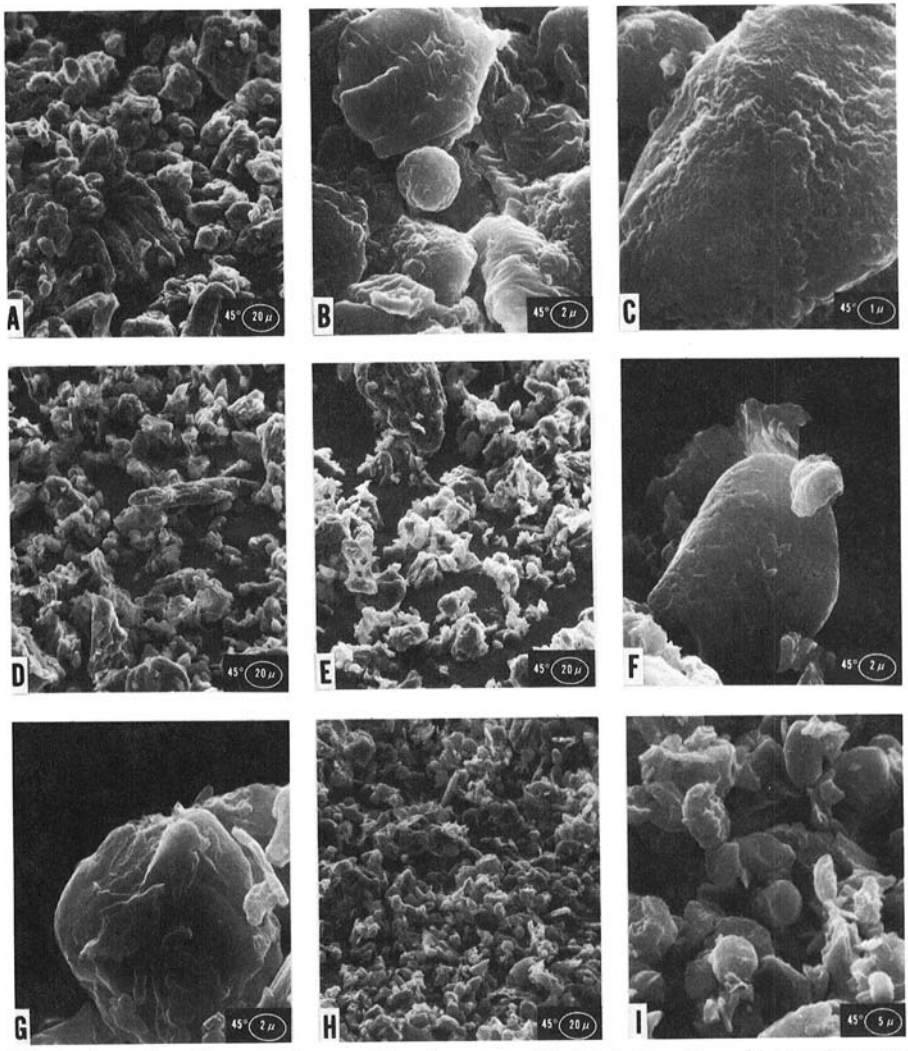

Fig. 2. Scanning electron micrographs of commercial full-fat soy flour (**A**, **B**, **C**); white defatted flour at low magnification (**D**); toasted defatted flour at low magnification (**E**); white defatted flour at high magnification (**F**); toasted defatted flour at high magnification (**G**); and a specially processed soy flour product containing 56% protein (**H**, **I**).

suggest that the meshlike network we observed earlier on the interior surfaces of cell walls (1) is a portion of the cytoplasmic network rather than a part of the cell wall.

The ease with which the contents were washed out of the cell sections indicates that water quickly dissolves the cytoplasmic network, and probably the protein bodies, with the result that the spherosomes are readily dislodged and removed. The water washings were noticeably turbid because of the spherosomes, and centrifugation (10 min at 12,000 $\times g$) yielded a fatty layer that floated on the top of the supernatant.

An additional feature observed in Fig. 1I is partial separation of cell walls at a juncture of three cells. The cell walls are clearly seen to be 0.5–1 μ thick.

Full-Fat Soy Flour

An edible product that closely resembles soybeans in composition is full-fat soy flour. Full-fat flours are prepared by steaming soybeans to inactivate lipoxygenase and other enzymes and to remove or destroy undesirable flavor compounds. After drying, the beans are dehulled and ground to a flour. Numerous spherical or elliptical particles 6–10 μ in diameter are observed in full-fat flour (Fig. 2A), and these, presumably, are protein bodies that survived the processing.

Higher magnification (Fig. 2B) did not readily reveal spherosomes as noted at the same magnification in the fracture surface (Fig. 1B). Still higher magnification, however, showed the presence of spherosomes on the surface of a protein body (Fig. 2C) but not as distinctly as observed earlier (Fig. 1B and 1C). It appears that some protein bodies and spherosomes remain intact during commercial conversion of soybeans into full-fat flour. In contrast, Saio and Watanabe (7) observed that the spherosomes coalesced into large oil droplets and that the protein bodies burst with curdling of the protein when water-soaked beans were steamed at 115° C for 30 min. These conditions, however, are likely to be more severe than those used commercially in the preparation of full-fat flour. The sample we examined did not contain the angular particles reported for extrusion cooked full-fat flour (2) presumably because of the differences in processing.

Defatted Flours

We examined three defatted flours representative of the range of moist heat treatments used by one manufacturer in the processing of these products. The flours and their typical nitrogen solubility indexes (NSI) were as follows: a) white flour (NSI 60–70); b) cooked flour (NSI 30–40); and c) toasted flour (NSI 15–25). All samples were 200 mesh in particle size. Comparison of the three flours indicated that the greater the extent of heat treatment the higher the degree of disruption of the protein bodies. The white flour (Fig. 2D) showed numerous intact protein bodies whereas the toasted flour (Fig. 2E) contained fewer particles that were clearly identified as protein bodies. The cooked flour was intermediate in appearance. Higher magnification of particles believed to be protein particles showed no significant differences between the white flour (Fig. 2F) and the toasted flour (Fig. 2G). Particles from both flours were covered with amorphous material; the protein network found in the defatted fracture surface (Fig. 1E and 1F) was not identifiable. Apparently this structural feature is

obliterated at some stage of processing.

We also examined a specially processed soy flour product (56% protein) which is used as a protein additive in bread baking (8). Photomicrographs (Fig. 2H and 2I) revealed a greater similarity to white flour (Fig. 2D) than to cooked flour (Fig. 2E). Numerous particles that appeared to be intact protein bodies were observed, but as noted with the other flours, the cytoplasmic network on the protein body surface (Fig. 1E and 1F) was not detected at high magnification.

Protein Concentrates

Protein concentrates prepared by the following three processes were examined: a) alcohol leaching (9); b) dilute acid leaching (10); and c) moist-heat treatment-water leaching (11). At low magnification the three products differed distinctly from each other (Fig. 3A-3C). The alcohol leached product (Fig. 3A) contained particles ranging from about 5 μ up to 80 μ in diameter. Some of the small particles appeared to be protein bodies (Fig. 3D) that remained intact during the leaching and drying process.

Protein concentrate prepared by dilute acid leaching consisted of partially collapsed spheres plus larger contorted particles (Fig. 3B). Closer examination revealed that the contorted particles are covered with a continuous film (Fig. 3E). Probably these particles are collapsed droplets formed during spray drying of the neutralized concentrate. The soluble proteins presumably form the continuous film on the collapsed droplets. Figure 3E shows two collapsed droplets that apparently collided and adhered to each other during drying. Also note the smaller (0.5–5 μ in diameter) collapsed droplets on the surface of the two large particles.

The protein concentrate made by moist-heat treatment followed by water leaching contained much larger particles (Fig. 3C) than the other two concentrates. Higher magnification failed to show residual structure of the cotyledon but suggested some fibrous texture (Fig. 3F).

Protein Isolates

We examined two isoelectric protein isolates (designated samples 1 and 2) and four sodium proteinates (samples 3–6). Processes for the preparation of these two forms of isolates are described elsewhere (12). The two isoelectric samples differed in particle size distribution and in their surface characteristics. Sample 1 (Fig. 3G) contained particles from 2 μ to more than 40 μ in diameter that were nearly spherical and had slightly rough surfaces. Although sample 2 (Fig. 3H) contained particles of about the same size range as sample 1, there were more large ones than small ones. The surfaces of the particles in sample 2 were much rougher than in sample 1 and some of the particles in sample 2 had holes in the surface. Higher magnification of sample 2 (Fig. 3I) showed that the particles are aggregates of much smaller particles; incomplete coalescence of the small particles probably causes the surface roughness.

The sodium proteinate forms of the isolates, like the isoelectric protein types, differed in their appearance as observed in the scanning electron microscope (Fig. 4). The particle shapes in isolate samples 3–6 ranged from spheres to partially collapsed spheres. Samples 3 (Fig. 4A) and 4 (Fig. 4B) were mixtures of spheres and partially collapsed spheres but sample 3 was more clumped together than sample 4. Samples 5 (Fig. 4C) and 6 (Fig. 4D) contained largely collapsed

spheres. Higher magnification shows that the particles have a smooth surface (Fig. 4E) which may be indented (Fig. 4F–4H) and frequently the large particles have small particles of similar shape adhering to them (Fig. 4F and 4G). Occasionally, one also sees broken spheres (Fig. 4B and 4I) that reveal a partially hollow structure. The outer covering of these broken particles is up to 2 μ thick and their interior consists of smaller particles that also appear to be collapsed spheres (Fig. 4I).

The marked distinctions between isoelectric isolates (Fig. 3G–3I) and the proteinates (Fig. 4) reflect the differences in conditions under which both protein

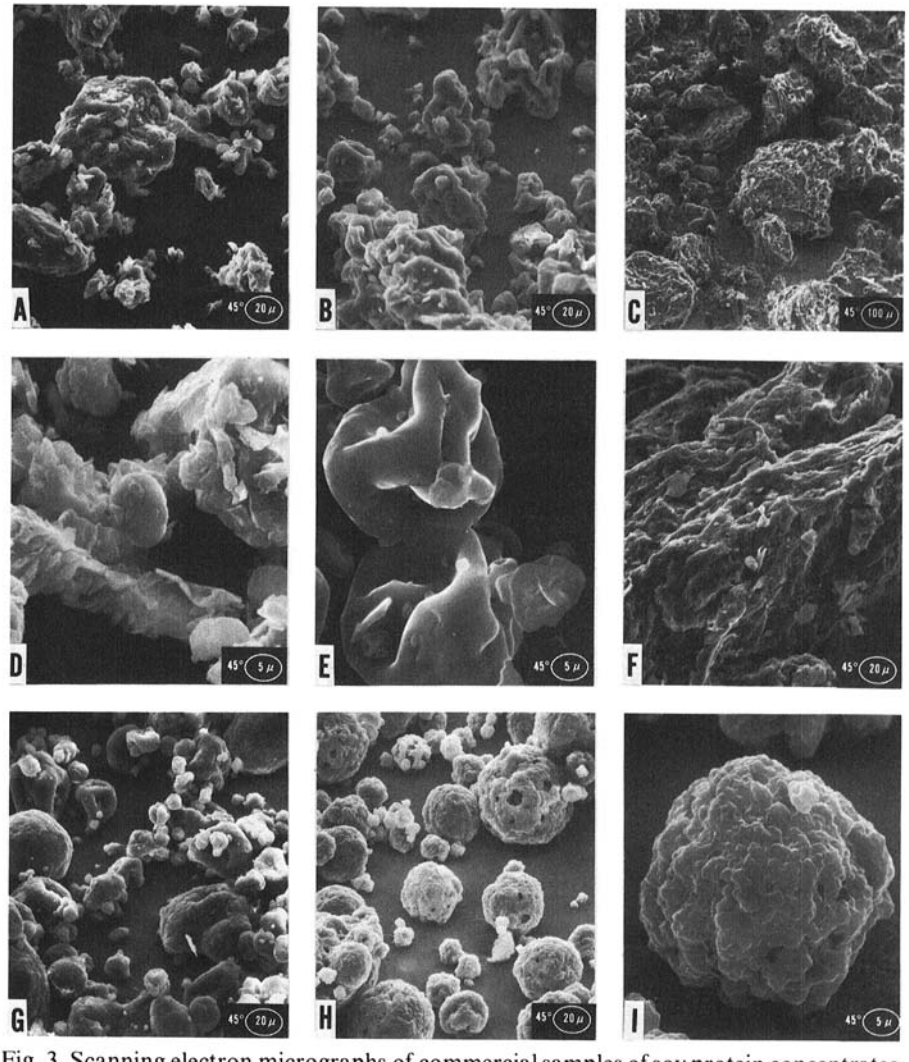

Fig. 3. Scanning electron micrographs of commercial samples of soy protein concentrates at low magnification (**A**, **B**, **C**); protein concentrates at high magnification (**D**, **E**, **F**); isoelectric protein isolate, sample 1 (**G**); and isoelectric protein isolate, sample 2 (**H**, **I**).

forms are spray dried (12). The isoelectric isolates are dried as insoluble dispersions that result in rough particles whereas the proteinates are dried from a more soluble form that yields smooth, partially hollow particles. Variations between isolates of a given type probably are the result of differences in processing history such as protein concentration and temperatures during spray drying. Collapsed spheres noted in the sodium proteinates resemble in appearance spray-dried calcium caseinate particles. Implosion of the caseinate particles (13) and uneven shrinkage of the casein (14) have been suggested as causes of the collapsed appearance.

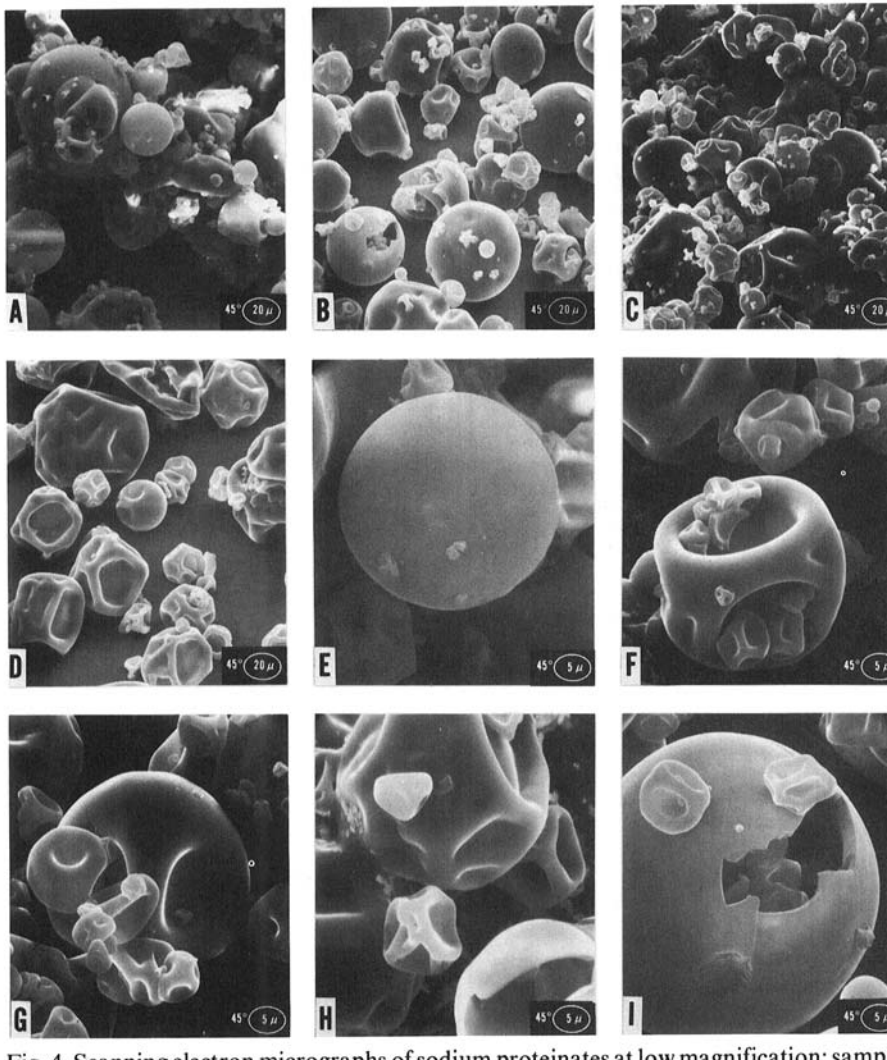

Fig. 4. Scanning electron micrographs of sodium proteinates at low magnification: sample 3 (**A**); sample 4 (**B**); sample 5 (**C**); and sample 6 (**D**). High magnification: sample 3 (**E**); sample 4 (**F**); sample 5 (**G**); sample 6 (**H**); and broken particle in sample 4 (**I**).

Significance of Results

The technique of freeze-fracturing (6) has revealed the internal structure of soybean cotyledon cells more clearly than previously observed by scanning electron microscopy of a mechanically fractured surface (1). The ease with which the contents of the cotyledon cells are washed out with water has not been demonstrated before. This behavior likely plays an important role in the traditional oriental preparation of soy milk. In this process soybeans are soaked in water, ground in a stone mill, and then heated (15). Grinding is undoubtedly a key step because once the cell wall is ruptured, extraction is very rapid and complete (Fig. 1G). Because the cell wall does not appear to rupture as a result of wetting, mechanical rupture is probably a prerequisite for efficient extraction.

Our results also indicate that some of the structural elements of the intact soybean cotyledon cell survive commercial processing. For example, protein bodies are detected in the flours and in some types of concentrates. It is likewise evident that new structural elements may form during processing. The most obvious examples of this type are the isolates, particularly those of the proteinate form. In these products, the soluble protein forms a smooth, continuous film as the water evaporates when the neutralized proteinate solution is spray dried.

It is apparent from our studies that the scanning electron microscope is a valuable research tool for detecting changes in particle size and shape when soybeans are processed into flours, concentrates, and isolates.

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